GENETIC POLYMORPHISM OF ALCOHOL DEHYDROGENASE 3 IN ALCOHOL LIVER CIRRHOSIS AND IN ALCOHOL CHRONIC PANCREATITIS

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Abstract — Aim: To find the ADH3 genotypes in the Polish population likely to be responsible for higher susceptibility to alcohol disease of the liver and chronic alcohol pancreatitis. Method: The ADH3 genotype and ADH3*/1 and ADH3*/2 alleles frequencies were examined in 198 patients. Genotyping of the ADH3 was performed using PCR-restriction fragment length polymorphism methods on a white cell DNA. Results: The genotype ADH3*/1/ADH3*/1 was found to be significantly more frequent in alcohol abusers compared with non-drinkers. The examinations of the group of alcohol abusers showed that the genotype ADH3*/2/ADH3*/2 occurred statistically significantly less frequently in patients with chronic pancreatitis than in those without alimentary lesions (healthy drinkers). The alleles ADH3*/1 and genotype ADH3*/1/ADH3*/1 were significantly more frequent in men than in women, whereas alleles ADH3*/2 and genotype ADH3*/2/ADH3*/2 were more common in women. Conclusions: The genotype ADH3*/2/ADH3*/2 is likely to be a protective factor for chronic pancreatitis. Variations in ADH3 genotypes may account for some of the differences in prevalence of alcohol dependence between genders in the Polish population.

INTRODUCTION

The abuse of alcohol leads to the damage of many organs: the liver, pancreas, gastric mucous membrane, cerebral tissue and results in the loss of behaviour control.

In Poland, people with alcohol dependence syndrome account for ~3% of the population (Akolinski, 1990). Cirrhosis develops in 10–20% of alcohol-addicts, chronic pancreatitis in 5%, and both in 1% (Czech and Hartleb, 2003).

The main alcohol metabolism occurs in the liver. One of the most important enzymes oxidizing ~80–90% of ethyl alcohol is alcohol dehydrogenase (ADH) (Groppi, 1990). This enzyme is encoded by seven genes located on the 4th chromosome; it exhibits high polymorphism and in humans occurs in over 20 isoenzymes which owing to structural and functional differences, kinetic properties and distribution in the organism were divided into five classes (Crabb et al., 1993; Agarwal, 2001). The class I isoenzymes are located at three gene loci: ADH1, ADH2, ADH3 and generate subunits α, β, γ, respectively (Chambers, 1990). The genes ADH2 and ADH3 exhibit polymorphism thereby are present in more than one form, encoding various forms of subunits β and γ of different properties (Day et al., 1991; Crabb et al., 1993). ADH3 forms two alleles ADH3*/1, ADH3*/2 encoding subunits γ1 and γ2, respectively. The subunit γ1 shows higher ethanol activity than the subunit γ2 (Day et al., 1991; Crabb et al., 1993; Szczechanek, 1999).

Genetic polymorphism of enzymes involved in alcohol metabolism plays a relevant role in etiopathogenesis of alcohol disease. This hypothesis suggesting the effects of ADH polymorphism was first put forward by Stomatoyannopoulus (1975). The alcoholic disease of the liver does not develop in all drinking individuals. Even in those consuming large amounts of alcohol systematically, the liver damage occurs in 77% of cases (Sherlock, 1995). It is assumed that this fact is associated with genetically determined differences in alcohol metabolism (Muramatsu et al., 1995; Chen et al., 1996; Whitfield, 1997; Ciczo-Lach, 2004).

It is important, however, that the presence of polymorphic isoenzymes ADH3 varies in different ethnic groups. ADH3*/1 occurs in 50–60% of Caucasians and in >90% of Asian population (Bosron and Li, 1986; Agrawal and Geodde, 1990).

There are few studies evaluating ADH polymorphism in the European population. The papers published deal mainly with oriental races.

The aim of the present study was to find in the Polish population the ADH3 genotypes, which are likely to be responsible for higher susceptibility to alcohol disease of the liver and chronic alcohol pancreatitis. An attempt was made to answer the following questions: are there any differences in the ADH3 genotype and alleles in the Polish population comparing patients addicted to alcohol with cirrhosis, chronic alcohol pancreatitis, those addicted but without liver and pancreas damage and non-drinkers?

MATERIALS AND METHODS

Materials

The study encompassed 198 patients: 48 women and 150 men. Average age was 45 ± 9.44 years.

Group I included patients with chronic alcohol abuse: 57 patients with alcohol liver cirrhosis (group IA), 44 patients with alcohol chronic pancreatitis (group IB), and 43 patients abusing alcohol but without damage to gastrointestinal organs—healthy drinkers (group IC).

Group II consisted of 54 non-drinking patients with functional alimentary disorders as a control group.

All the patients gave informed consent for examinations.

Group I included patients consuming on average >80 g of pure ethanol a day for at least 2 years, average 160.86 ± 43.93 g daily. All the patients examined had positive CAGE test (Steinweg and Worth, 1993; Ewing et al., 1998). The diagnosis of
cirrhosis and chronic pancreatitis was based on the generally accepted criteria (Brzozowski, 1991; Dzieniszewski et al., 2004). Other non-alcoholic causes of liver cirrhosis and chronic pancreatitis were excluded. The patients with alcoholic disease without alimentary organ damage (group IC) underwent the examinations to exclude alimentary pathology, particularly of the liver and pancreas.

Group II consisted of patients in whom the history allowed to confirm that they did not drink alcohol, the organic alimentary pathology was excluded, and functional disorders were diagnosed.

METHODS

The ADH3 genotype and the frequency of ADH3 alleles were determined in all the patients.

Genotyping of the ADH3 (exon VIII) was performed using PCR—restriction fragment length polymorphism methods on a white cell DNA. The primers for amplification were: ADH3 321 (5’-GCTTAAAGAGTAAATATCTGTCCC-3’) and ADH3 351 (5’-AACCCCATCCTTGCAGAGC-3’) (Groppi et al., 1990). The PCR total reaction mixture of 25 μl contained: 0.6 μg of genomic DNA, 0.2 μM of each primer, 0.2 mM of each dNTP, 2.5 mM of MgCl2, 1× PCR buffer with (NH4)2SO4 and 0.5 U of Taq DNA Polymerase (MBI Fermentas). The PCR reaction was realized in Mastercycler personal (Eppendorf AG, Germany) under the following conditions: initial denaturation at 95°C for 5 min; then 35 cycles of denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s, extension at 72°C for 30 s; and 1 cycle of final extension at 72°C for 10 min.

The amplified product was digested with 10 U of enzyme SspI (MBI Fermentas) per 20 μl of reaction mixture at 37°C overnight, and subjected to electrophoresis in 3% agarose gel (or 12% polyacrylamide gel), stained with ethidium bromide.

Statistical analysis

The genotypes and alleles were compared using the χ² homogeneity test. The calculations were performed by STATISTICA PL software (Oktaba, 1996; Jóźwiak and Podgórska, 1998; Stanisz, 1998).

RESULTS

Tables 1 and 2 present the presence of ADH3 alleles and ADH3 genotype in the groups examined.

The examinations of the presence of allele ADH3*1 among alcohol abusers showed that alleles ADH3*1 were more frequent in patients with cirrhosis hepatitis and chronic pancreatitis than in patients without alimentary organ damage (healthy drinkers). But differences were no statistically significant (P > 0.05).

The allele ADH3*1 was found to be more statistically significantly frequent in alcohol abusers than non-drinkers (P < 0.001). Alleles ADH3*1 were more statistically significantly frequent in patients with alcohol cirrhosis hepatitis and alcohol chronic pancreatitis than in non-drinkers (P < 0.001).

DISCUSSION

Genetic factors are strongly involved in the development of alcohol addition. They determine the individual susceptibility to alcoholism and alcoholic damage to the alimentary tract. It seems, however, that whether the genetically predisposed individual becomes an alcoholic or not is determined by interactions between genetic factors and promoting or protecting environmental effects (Schuckit, 1995). It would be important to find the genes responsible for the susceptibility to alcohol addiction and to conduct the screening examinations defining whether a particular individual is genetically loaded, which might reduce this phenomenon. The genetically loaded individuals would be able to choose...
consciously between increased risk of alcohol consumption and abstinence.

In the Caucasians, the ADH3*1 alleles are found in 50–60% of the population and their influence on the development of alcoholic hepatic damage varies in the European inhabitants.

In the Polish population, according to Kwast (1983), the frequency of ADH3*1 was 60.1%, whereas ADH3*2 was 39.9%. This frequency was calculated on the basis of phenotype studies. And there are no epidemiological data about the death causes and alcohol consumption in the group examined.

Borras et al. (2000) evaluated ADH2 and ADH3 polymorphism in the European population consisting of 876 individuals from five countries: Spain, France, Germany, Sweden, and Poland (193 individuals). On average, the alleles ADH3*1 were observed in 55.6% of healthy non-drinking volunteers and ADH3*2 in 44.4%. In alcohol abusers with cirrhosis and without hepatic parenchyma, the damage was 57.7 and 42.3%, respectively. Similar results were obtained by French scientists assessing ADH3 in 46 alcoholics with cirrhosis and in 39 controls (Cozigou et al., 1990).

The study examining ADH polymorphism in the Spanish population demonstrated higher but not statistically significant frequency of ADH3*1 alleles in alcoholics than in non-drinkers (Espinosa et al., 1997). Another study evaluating 145 Spanish individuals showed no differences in ADH3 allele frequency with respect to alcoholic cirrhosis, non-alcoholic chronic liver damage and healthy subjects (Pares et al., 1994).

Our results demonstrate that the ADH3*1 allele is found in 62.3% of patients with alcoholic hepatic cirrhosis, in 68.2% of those with alcoholic chronic pancreatitis, and in 59.3% of alcohol abusers without alimentary damage; thus its frequency was slightly higher than that in the Borras et al. (2000) study. It should be noticed that in non-drinkers the presence of this allele is statistically significantly lower whereas that of ADH3*2 is higher. Therefore it may be thought that ADH3*1 promotes alcoholism whereas ADH3*2 is likely to be a protective factor in the Polish population. Likewise, the genotype ADH3*1/ADH3*1 is more common in alcohol abusers than in non-drinkers. It promotes alcoholic cirrhosis and chronic alcoholic pancreatitis.

The findings in the Asian population are different. The ADH3*1 allele in this population is more commonly spread, its frequency is estimated at 90%. The ADH3*1 allele encoding an extremely active subunit γ1 is rare in Asian alcohol abusers, although there are studies demonstrating no ADH3 polymorphism differences between Asian alcoholics and non-drinkers (Chao et al., 1997).

Similar studies conducted in the Chinese population of alcoholics without liver damage suggested lower frequency of ADH3*1 alleles compared with non-drinkers (Thomasson et al., 1991; Chao et al., 1994).

Mulligan et al. (2003) in their study examining ADH2, ADH3, ALDH2 polymorphisms in the population of American Indians addicted to alcohol stress their significant effects on the development of alcoholic disease. The authors suggest that the presence of ADH3*1 decreases the risk of alcohol addiction in this population. Another study examined ADH3 polymorphism in the population of 371 Kenyans and found no statistically significant differences in alleles and genotypes between alcohol addicts and non-drinkers (Orir et al., 2004).

It may be concluded that the role of ADH3 polymorphism is different in various races and varies markedly from one population to another. Coexisting environmental factors are also likely to be involved.

There are many reports evaluating the relation between alcoholic damage to other alimentary organs and ADH3 polymorphism. Drenth et al. (2001) demonstrated that ADH3 polymorphism may be the risk factor of chronic pancreatitis. Chronic pancreatitis was more common in ADH3*1 and ADH3*2 homozygotes than in heterozygotes. The authors concluded that the presence of heterozygotous alleles have a protective role in the etiology of chronic pancreatitis in the group examined (Drenth et al., 2001).

To date the studies in Caucasian patients have not been carried out.

The results of our studies are different. In the group of patients with alcoholic pancreatitis higher frequency of the heterogenous ADH3*1/ADH3*2 genotype was observed whereas the presence of homozygotous ADH3*2/ADH3*2 was estimated at 2.3%. It may be thought that the possession of this genotype generally does not favour alcoholism and protects against chronic pancreatitis. However, ADH3*1 alleles evidently showed coexistence with alcoholic chronic pancreatitis.

The factor promoting alcohol addiction is sex. In the general population, alcoholism is eight times more frequent in men than in women (Wilsnack et al., 1997). However, the unfavourable effects of ethanol on the female organism are revealed quicker and at lower doses of alcohol consumed.

In the study involving 183 Caucasians a significantly higher frequency of ADH3*1 was observed in men (Szczepanek, 1999). This is in agreement with our results, which confirm higher frequency of ADH3*1 allele in men compared with women. A statistically significant low frequency of the ADH3*1 allele in women and higher frequency of ADH3*2 are also likely to be protective factors decreasing the risk of ethanol addiction.

The influence of genetic ADH3 polymorphism on the development of alcohol addiction, alcoholic cirrhosis, or alcoholic pancreatic damage should be considered in terms of individual races. The presence of ADH3 alleles and genotypes is

### Table 4. The presence of ADH3 genotypes and ADH3 alleles in men and women

<table>
<thead>
<tr>
<th>Genotype</th>
<th>ADH3<em>1/ADH3</em>1</th>
<th>ADH3<em>1/ADH3</em>2</th>
<th>ADH3<em>2/ADH3</em>2</th>
<th>ADH3*1</th>
<th>ADH3*2</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>71 (41.0%)***</td>
<td>74 (42.8%)**</td>
<td>28 (16.2%)***</td>
<td>216 (62.4%)***</td>
<td>130 (37.6%)***</td>
</tr>
<tr>
<td>F</td>
<td>10 (10.7%)***</td>
<td>45 (48.4%)**</td>
<td>38 (40.9%)***</td>
<td>65 (35.0%)***</td>
<td>121 (65.0%)***</td>
</tr>
</tbody>
</table>

***P < 0.001.
**P > 0.05.
different in the Caucasian population and in Asians. The factors which seem to protect against alcoholism and alcoholic pathologies of the gastrointestinal tract in the Polish population play a different role in other races and populations.

Our studies suggest that in the Polish population examined the ADH3*1 allele and ADH3*1/ADH3*1 genotype occur more frequently in alcohol abusers and promote alcoholism, alcoholic cirrhosis, and chronic pancreatitis, whereas the ADH3*2 allele and ADH3*2/ADH3*2 genotype may protect against alcohol abuse. Among the patients consuming excessive amounts of alcohol, the ADH3*2/ADH3*2 genotype is extremely rare in chronic pancreatitis individuals; thus it is likely to be the protective factor of this disease.

The ADH3*1 allele and ADH3*1/ADH3*1 genotype are more common in men, whereas the ADH3*2 allele and ADH3*2/ADH3*2 genotype are present more frequently in women. This may account for rarer alcohol addiction observed in women.

However, generalizations should be made cautiously; it ought to be kept in mind that the role of genetic ADH3 polymorphism in the development of alcoholic alimentary damage is different in different populations, in which some other alcoholism promoting or protecting factors may be present. These factors are likely to be coexisting genetic polymorphisms in other genes encoding enzymes involved in ethanol metabolism. Further studies will deal with their examination.

REFERENCES


